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Original Article

Chemical composition of essential oil and hexane extract and antioxidant activity of various extracts of *Acmella uliginosa* (Sw.) Cass flowers from Indonesia

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ABSTRACT

Medicinal plants are rich sources of natural antioxidant which are used in the prevention and treatment of disease like artherosclerosis, heart stroke, diabetes and cancer and to delay the process of aging. *Acmella uliginosa* (Sw.) Cass is an edible herb traditionally used in the treatment of many diseases. Analysis of volatile components in the flower extract used gas chromatography-mass spectrometry. The results showed the main components of the essential oil were caryophyllene (21.27%), caryophyllene oxide (15.49%), and 3-carene (10.73%). The main components of the hexane extract were N-isobutyl-2E,6Z,8E-decatrienamide (37.80%), α-pinene (4.98%) and hexadacanoic acid-methyl ester (4.78%). The antioxidant activity of *A. uliginosa* (Sw.) Cass flower from Indonesia was determined using 1,1, diphenyl-2-picryl hydrazine (DPPH) free radical scavenging assay. The IC_{50} (defined as the total antioxidant necessary to decrease the initial DPPH radical by 50%) of extracts was calculated. A comparative study determined that *A. uliginosa* (Sw.) Cass in methanol extract showed higher antioxidant potential (IC_{50} = 96.83 μg/mL) compared to ethyl acetate extract (IC_{50} = 123.46 μg/mL) and n-hexane extract (905.92 μg/mL) against DPPH free radicals.

Introduction

The genus *Acmella* (Family Asteracea) comprises 30 species including *Acmella uliginosa* (Sw.) Cass and nine additional infra-specific taxa that are mainly distributed in the tropical and sub-tropical regions around the world, such as Indonesia (Chung et al., 2008). The whole aerial parts, flower heads and roots yield a compound known as spilanthol amide which has a saliva-inducing effect as well as being a powerful insecticide and local anaesthetic (Sana et al., 2010). Chemicals in plants are rich sources of secondary metabolites like flavonoids, flavones, isoflavones, antocyanins, catechins, polyphenols and many of these chemical compounds posses biological activity with antimicrobial and antioxidant properties (Sana et al., 2010). Although a large number of species of *Acmella* are being used ethnomedically for different kind of diseases throughout the world, very little chemical or biological investigation has been done on *A. ulliginosa* (Ahmed et al., 2012). The flower heads are reported to be chewed to relieve toothache and other mouth-related troubles (Rao et al., 2012). They are also used as a preventive medicine for scurvy and to stimulate digestion, the flower heads have been used as a spice for appetizers by the Japanese and its extract is used as a flavoring material for dentifrices and gum (Leng et al., 2011). Leaves are used externally in the treatment of skin disease and a leaf decoction is used as a diuretic and lithotriptic, while the whole plant is used in the treatment of dysentery (Leng et al., 2011; Rao et al., 2012).

Phytochemical analysis is essential to make good use of any medicinal plant and to establish a relation between the pharmacology and the chemistry of the plant. Many studies have been done on the chemical analysis and structure determination of pungent alkamides from *A. uliginosa* and the major pungent compound is spilanthol, which is an isobutylamide and is well known for its insecticidal properties (Leng et al., 2011). Plant-extracted phytochemicals have been used in nutraceuticals, pharmaceuticals, herbal medicines, spices, insect repellents, cosmetics, perfumes and many other beneficial secondary metabolites (Dubey et al., 2013).

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Recently, health foods, herbs and dietary supplements enriched with medicinal ingredients such as antioxidants and bioactive metabolites have drawn considerable attention worldwide, especially herbs that are used in food and traditional medicines (Prachayasittikul et al., 2013). Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular disease and inflammatory diseases (Soni and Sosa, 2013). Lawrence and Lawrence (2011) defined the term 'antioxidant' as the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by reactive oxygen species (ROS). The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals (Lawrence and Lawrence, 2011; Padmanabhan, 2012). Food antioxidants play an important role in the food industry due to their ability to neutralize free radicals that might be generated in the body by donating their own electrons to free radicals without becoming free radicals themselves (Msagati, 2013).

The 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical method is an antioxidant assay based on electron transfer that produces a violet solution in ethanol and this free radical, stable at room temperature is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution; the use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry, so it can be useful to assess various products at a time (Garcia et al., 2012; Subedi et al., 2012). The objectives of this study were to analyze the chemical composition of the essential oil and hexane extract and to evaluate the antioxidant activity of various extracts of *A. uliginosa* (Sw.) Cass flowers.

Materials and methods

Sample preparation

Fresh flowers of *A. uliginosa* (Sw.) Cass were collected in Ciparay, Bandung in West Java, Indonesia. Authentication of plant species was carried out by Dr. J.S. Rahajoe in the Herbarium Bogoriense, Indonesia.

Isolation of essential oil

A sample of 100 g of fresh flowers of *A. uliginosa* (Sw.) Cass was extracted using a Clevenger distillation apparatus with a ratio of sample to water of 1:6 over a 12 h period. The essential oil was separated from the aqueous solution, dried over anhydrous Na_2SO_4 and then collected and kept in a light-protected bottle. The essential oil was investigated for its chemical composition.

Sample extraction

A sample of 250 g of fresh *A. uliginosa* flowers was macerated in 1000 mL of solvent (methanol, ethyl acetate and normal hexane) for 5 d. The results of the extraction were filtered, condensed using a rotary evaporator and used for analysis.

Gas chromatography–mass spectrometry analysis

Essential oil and hexane extract of *A. uliginosa* were identified using gas chromatography-mass spectrometry (GC–MS). Components were identified by matching their mass spectra with those recorded in the mass spectral library. The GC–MS analyses were performed using the following equipment from Agilent Technologies (Santa Clara, CA, USA): HP7890 GC System; 5975C series GC MSD; GC 7890 with FID detector; FID heater at 275 °C, HP-1 column with methyl siloxane (30 m × 250 μm × 0.25 μm film thickness); helium carrier gas pressure, 13,957.74 N/m^2; inlet:split ratio, 500:1; total flow, 53.1 mL/min; septum purge flow, 3 mL/min; and gas saver on 20 mL/min after 2 min. The oven temperature was programmed from 100 °C for 10 min, then 5 °C/min to 200 °C for 0 min, then 10 °C/min for 10 min, for a total running time of 45 min. The multispectral acquisition parameters were: electron multiplier voltage (EMV), mode, relative; relative voltage, 0 V (V), resulting EMV, 2506 V; the scan parameters were: low mass, 30.0; high mass, 500.0; threshold, 50; and the MS zones were: MS source, 250 °C and MS quadrupole, 200 °C; ionization voltage, 70 eV; mass selective detector transfer line, 300 °C. Compound identification was done by comparing the Wiley 2008-National Institute of Standards and Technology (NIST) library and P. Adam data of the peaks with those reported in the literature and the mass spectra of the peaks with literature data.

Antioxidant activity

The antioxidant activity was evaluated using the free radical scavenging activity of the DPPH method. DPPH is free radical, but stable. The DPPH solution is initially violet in color which fades when antioxidants donate hydrogen. The change in color is monitored using a spectrophotometer and the DPPH free radical scavenging activity is calculated (Molyneux, 2004).

A stock solution of 0.1 mM DPPH in methanol was made. Test samples of extract were made at 20 μg/mL, 40 μg/mL, 60 μg/mL, 80 μg/mL and 100 μg/mL in methanol and ethyl acetate. Test samples of hexane extract were made at 100 μg/mL, 200 μg/mL, 300 μg/mL, 400 μg/mL and 500 μg/mL. The absorbance was measured at 517 nm using an ultraviolet visible spectrophotometer (Shimadzu Corp.; Kyoto, Japan). After 30 min, the percentage scavenging was calculated using Eq. (1):

$$\text{Percentage scavenging} = \frac{(A0 - AT)}{A0} \times 100\% \quad (1)$$

where $A0$ is the absorbance of the DPPH solution and AT is the absorbance of the test or reference sample. The percentage scavenging was then plotted against the concentration and a regression equation was obtained to calculate the IC_{50} which is defined as the total antioxidant necessary to decrease the initial DPPH radical by 50% (Saikia and Upadhyaya, 2011).

Results and discussion

Yield of essential oil and different extracts

The essential oil, which was a greenish yellow color, was obtained by hydro-distillation of the fresh flowers of *A. uliginosa*. The yield of the oil was 0.37% (volume per weight).

The yield of extract from *A. uliginosa* flowers in methanol, ethyl acetate and hexane was 2.03%, 1.09%, and 0.99%, respectively. Differences in the yield of extract from different solvents might be attributed to the availability of the extractable component of different polarities.

Chemical composition

The results of the GC–MS analysis of the essential oil revealed different phytochemical compositions (monoterpenoids, sesquiterpenoids, acyclic alkanes, oxygenated sesquiterpenoids, aromatic monoterpenoids, alkaloids, furan, and olefins) as shown in Table 1.

Fig. 1 shows the chromatogram from the GC–MS for the essential oil of *A. uliginosa* flowers. The essential oil of *A. uliginosa* flowers showed the presence of 44 organic compounds (Table 1).

Table 1
Chemical compositions of volatile oil of *Acmella uliginosa* (Sw.) Cass flowers.

No.	Chemical composition	Retention time (min)	Percentage (%)	Quality (%)	Chemical class
1	Alpha pinene	3.69	0.81	96	Monoterpenoids
2	Sabinene	4.16	2.30	97	Monoterpenoids
3	Beta pinene	4.29	7.32	97	Monoterpenoids
4	Limonene	4.66	0.59	93	Monoterpenoids
5	Para cymene	4.96	0.64	95	Aromatic monoterpenoids
6	3-Carene	5.18	10.73	92	Monoterpenoids
7	Beta ocimene	5.44	0.17	98	Monoterpenoids
8	6-Methyl-3,5-heptadien-2-one	6.49	0.02	87	Monoterpenoids
9	Alpha naginatene	6.60	0.12	94	Furans
10	2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	7.95	0.40	97	Acyclic olefins
11	4-Isopropylcyclohex-2-en-1-one	9.61	1.39	95	Cyclic ketones
12	1-Isopropyl-4-methyl-3-cyclohexen-1-ol	9.94	1.31	98	Monoterpenoids
13	Propanal, 2-methyl-3-phenyl-	12.31	0.16	98	Aromatic monoterpenoids
14	2-Methoxy-4-(1-methylethyl)toluene	12.48	0.16	95	Aromatic monoterpenoids
15	(4-Isopropylphenyl) methanol	14.53	0.93	91	Aromatic monoterpenoids
16	2,4-Diisopropenyl-1-methyl-1-vinyl cyclohexane	19.09	0.65	99	Sesquiterpenoids
17	3H-3a,7-Methanoazulene, 2,4,5,6,7,8-hexahydro-1,4,9,9-tetramethyl-,[3aR-(3a.alpha.,4.beta.,7.alpha.)]	19.43	0.17	95	Sesquiterpenoids
18	Caryophyllene	20.04	21.27	99	Sesquiterpenoids
19	Alpha bergamotene	20.53	0.49	95	Monoterpenoids
20	Humulene	20.98	2.38	99	Sesquiterpenoids
21	1,10-Undecadiene	21.62	1.51	83	Aliphatic unsaturated (olefins)
22	Germacrene D	21.74	0.21	99	Sesquiterpenoids
23	Beta farnesene	21.86	0.39	99	Sesquiterpenoids
24	1-Tetradecene	22.26	1.32	91	Aliphatic olefins
25	Beta bisabolene	22.52	0.30	98	Sesquiterpenoids
26	Naphthalene, 1,2,3,4,5,8-hexahydro-6-methoxy-7-methyl-1-(1-methylethyl)-, (.+-.)-	23.73	1.81	90	Sesquiterpenoids
27	Caryophyllene oxide	24.30	15.49	95	Oxygenated sesquiterpenoids
28	3-Isopropyl-6,8a-dimethyl-2,3,4,5,8,8a-hexahydro-3a(1H) azulenol	24.73	2.21	93	Sesquiterpenoid alcohols
29	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)]-	24.90	0.91	83	Oxygenated sesquiterpenoids
30	Isoaromadendrene epoxide	25.46	0.59	83	Oxygenated sesquiterpenoids
31	Vulgarol A	25.56	0.59	80	Diterpenoid alcohols
32	Bicyclo[4.4.0]dec-1-en, 2-isopropyl-5-methyl-9-methylene	25.69	0.61	90	Sesquiterpenoids
33	Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-, (S)-	25.79	0.36	96	Sesquiterpenoids
34	4-Isopropyl-1,6-dimethyl-1,2,3,4,4a,7,8,8a-octahydro-1-naphthalenol	25.98	0.83	87	Sesquiterpenoid ketones
35	(-)-5-Oxatricyclo[8.2.0.0(4,6)] dodecane,12-trimethyl-9-methylene-,[1R-(1R*,4R*,6R*,10S*)]-	26.35	1.12	83	Oxygenated sesquiterpenoids
36	Bicyclo [10.1.0]tridec-1-ene	26.47	0.25	93	Cyclic olefins
37	(4S,5R)-5-Hydroxycaryophyll-8(13)-ene-4,12-epoxide	28.48	0.19	90	Oxygenated sesquiterpenoids
38	2-Pentadecanone, 6,10,14-trimethyl-	30.33	0.29	91	Aliphatic ketones
39	N-Isobutyl-2(E),6(Z),8(E)-decatrienamide	30.54	0.98	90	Alkaloids
40	(4S,5R)-5-hydroxycaryophyll-8(13)-ene-4,12-epoxide	31.76	0.08	90	Oxygenated sesquiterpenoids
41	Trans phytol	34.43	0.38	90	Acyclic diterpenoids (prenol lipids)
42	Eicosane	35.61	0.06	97	Acyclic alkanes
43	Tricosane	36.84	0.11	99	Acyclic alkanes
44	Octadecane	39.99	0.17	97	Acyclic alkanes

The six major phytocompounds identified were caryophyllene (21.27%), caryophyllene oxide (15.49%), 3-carene (10.73%), beta pinene (7.32%), humulene (2.38%) and sabinene (2.30%). The other compounds were present in minor percentages.

Caryophyllene is useful in various applications such as odor and taste-modifying agents, potential anticarcinogenic agents, the control of whitefly species, a repellent for pine wood nematodes and as a component of antitumor compositions (Pichette et al., 2004; Pianowski et al., 2008). Caryophyllenes, more particularly alpha humulene or beta caryophyllene, are used as an anti-inflammatory and as an analgesic, in a broad sense (Bakir et al., 2008). The compounds were found to be useful inhibitors of entities that are known to be involved in the inflammatory process: pro-inflammatory cytokines IL-1β (interleukin 1β) and TNFα (tumor necrosis factor α) according to Pianowski et al. (2008).

Caryophyllene oxide is useful as a fragrance ingredient to give woody, spicy characters and to enhance the aroma of a product (Safrudin, 2014). As a flavor, caryophyllene oxide is used in formulas of mint, savory vegetable, savory spices, fruity citrus, fruity red, fruity tropical and alcoholic beverages (Silva et al., 2012; Safrudin, 2014). Caryophyllene oxide has a wide pharmacological effect (Park et al., 2011). It also has ability as an antibacterial agent, and the following properties: anti-fungal, immune-modulation, anti-inflammation, anti-rheumatic, antioxidant, anticancer and anti carcinogenic (Park et al., 2011; Singh et al., 2014). Caryophyllene oxide is also known as a preservative in food, medicines and cosmetics (Yang et al., 1999). Caryophyllene oxide activity is comparable to standard medicinal ability, hence the potential of this compound to be used as an analgesic and in anti-inflammation drugs (Safrudin, 2014).

The results of the GC–MS analysis of hexane extract revealed different phytochemical compositions as shown in Table 2.

Fig. 2 shows the chromatogram from GC–MS for the hexane extract from *A. uliginosa* flowers. In total, 20 compounds (Table 2) were identified from the hexane extract of *A. uliginosa*. The three main compounds in the n-hexane extract were N-isobutyl-2(E),6(Z),8(E)-decatrienamide (37.80%), α-pinene (4.98%), and hexadecanoic acid-methyl ester (4.78%). The other compounds were present in minor percentages.

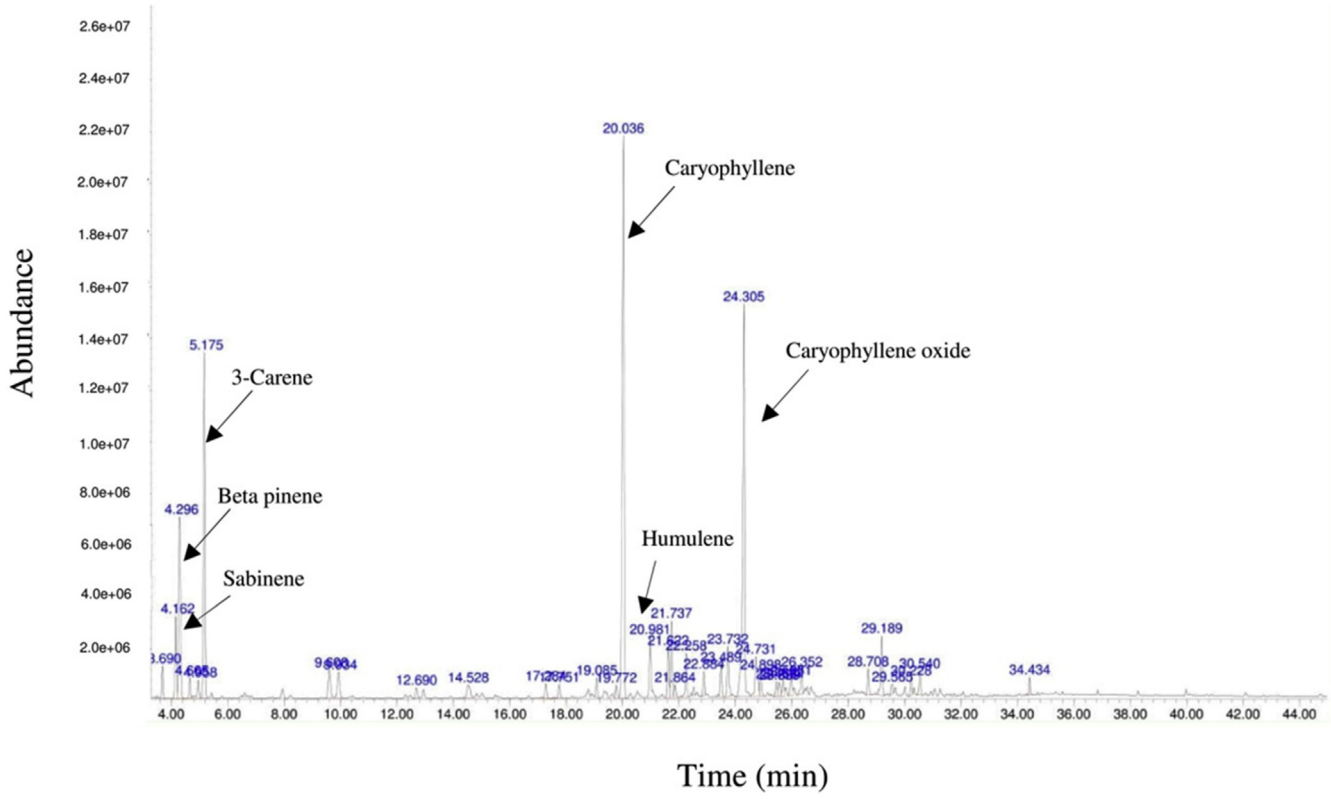

Fig. 1. Chromatogram of essential oil of *Acmella uliginosa* (Sw.) Cass flowers.

Table 2
Chemical compositions of hexane extract from *Acmella uliginosa* (Sw.) Cass flowers.

No.	Chemical composition	Retention time (min)	Percentage (%)	Quality (%)	Chemical class
1	Sabinene	4.571	0.47	99	Monoterpenoids
2	β-Myrcene	4.728	1.23	96	Monoterpenoids
3	α-Pinene	5.581	4.98	96	Monoterpenoids
4	2-Methoxy-4 vinylphenol	15.461	1.04	96	Phenol and derivatives
5	β-Caryophyllene	20.048	2.94	99	Sesquiterpenoids
6	β-Cubebene	21.766	1.05	98	Sesquiterpenoids
7	1-Pentadecene	22.279	0.81	99	Acyclic olefins
8	β-Caryophyllene epoxide	24.271	0.62	94	Oxygenated sesquiterpenes
9	N-Isobutyl-(6Z,8E)-decadienamide	29.431	0.37	90	Alkaloids
10	N-Isobutyl-2(E),6(Z),8(E)-decatrienamide	30.814	37.80	83	Alkaloids
11	Methyl 15-methylhexadecanoate	33.277	0.13	99	Fatty acid esters
12	Hexadecanoic acid methyl ester	32.845	4.76	99	Fatty acid esters
13	9,12-Octadecadienoic acid, methyl ester	34.098	2.47	99	Fatty acid esters
14	Linoleic acid, methyl ester	34.147	1.71	99	Fatty acid esters
15	N-Isobutylundeca-(2E,4E), diene-8,10-diynamide	34.249	1.95	99	Alkaloids
16	N-(4-Cyanophenyl) pyrrolidine-3-carbaldehyde	34.439	1.50	93	Heteroaromatics
17	Octadecanoic acid, ethyl ester	35.346	0.67	99	Fatty acid esters
18	Propanamide, 3-chloro-N-(2-phenylethyl)	36.378	0.63	81	Haloalkamides
19	N-(2-Phenylethyl) (2E,6Z,8E)-decatrienamide	37.631	4.28	96	Alkaloids
20	Pentacosane	39.986	1.21	98	Acyclic alkanes

The main compound in the hexane extract of *A. uliginosa* was alkamide (alkaloid compounds). The alkamide content was evaluated and a higher concentration was observed in the flower head. Many of the species that contain alkamides have been used in traditional medicines of different civilizations and they are known for their pungent taste and for causing itching and salivating (Chaves et al., 2003). Some species have been used in the treatment of toothache (Chaves et al., 2003; Dubey et al., 2013). One of the most widely distributed alkamides in these species that is responsible for various biological activities is N-isobutyl-2E,6Z,8E, decatrienamide (Chaves et al., 2003). N-Isobutyl-2E,6Z,8E, decatrienamide which is commonly named as spilanthol is a key chemical compound that can be concentrated in n-hexane extract, which was found to contain 38.96% spilanthol. Spilanthol has a strong pungent taste, it may produce local astringency and anaesthetic effects (Tiwari et al., 2011). More specifically, spilanthol (N-isobutyl-2,6,8, decatrienamide) is known to cause a smarting or numbing stimulus and a piercing stimulative feeling, and is used as a spice in foods and beverages (Tanaka et al., 2012). The (2E,6Z,8E) isomer is useful as a sense stimulus component in a wide range of product such as foods, beverages, fragrances and cosmetic (Tanaka et al., 2012).

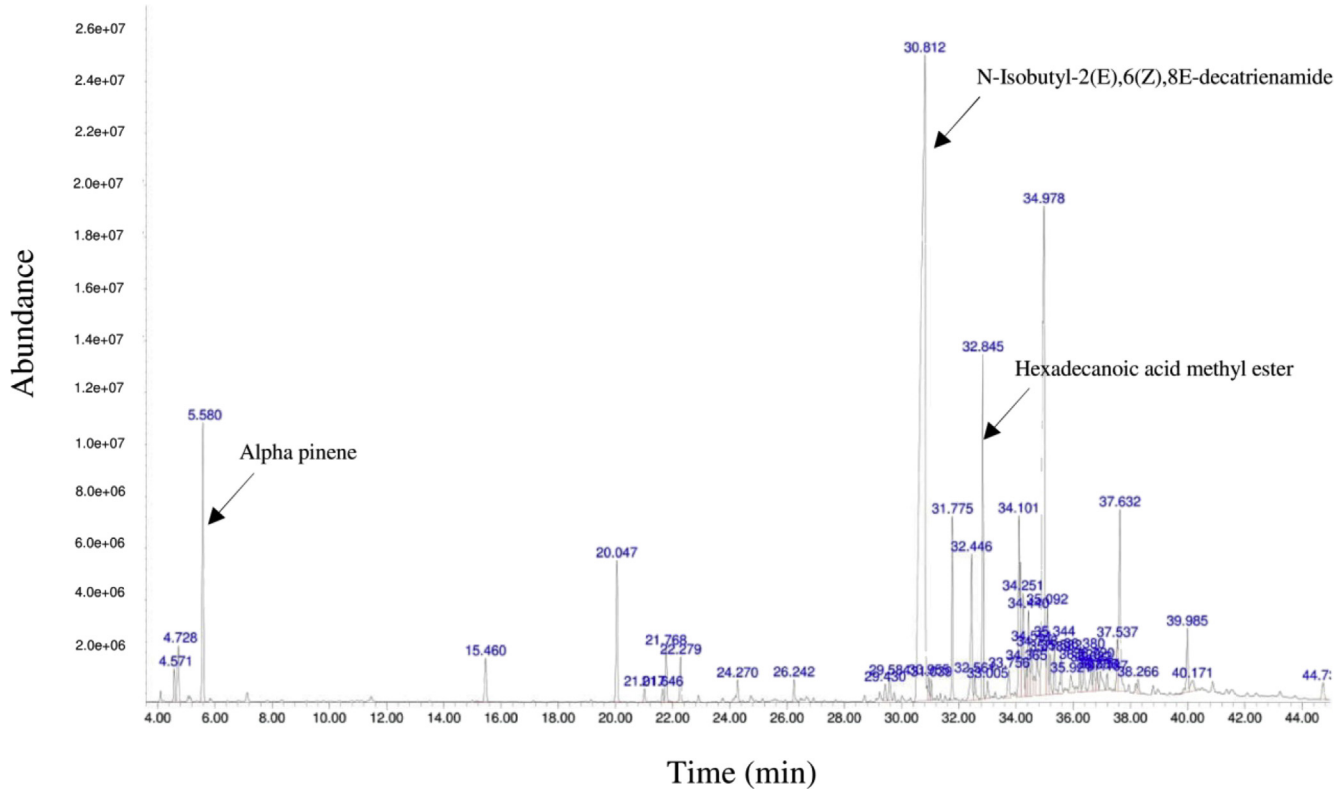

Fig. 2. Chromatogram of hexane extract of *Acmella uliginosa* (Sw.) Cass flowers.

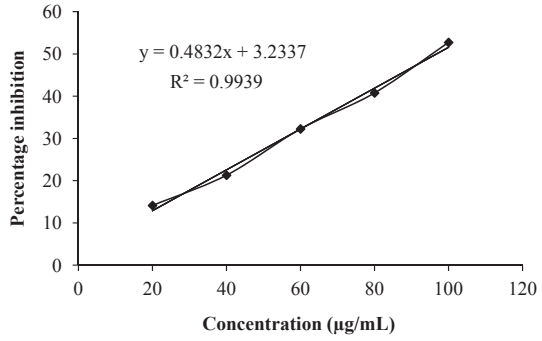

Fig. 3. Comparison of percentage inhibition of 1,1, diphenyl-2-picryl hydrazine at different concentrations in methanol extract (equation shown by dotted thin line; R^2 = coefficient of determination).

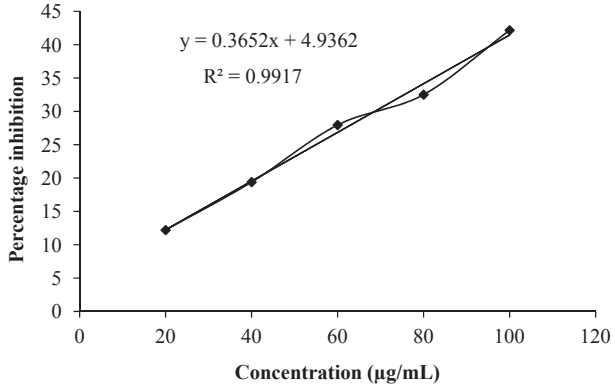

Fig. 4. Comparison of percentage inhibition of 1,1, diphenyl-2-picryl hydrazine at different concentrations in ethyl acetate extract (equation shown by dotted thin line; R^2 = coefficient of determination).

Antioxidant activity

The DPPH radical scavenging activity in methanol, ethyl acetate and n-hexane extract from *A. uliginosa* was recorded in terms of the percentage inhibition or percentage scavenging of DPPH as shown in Figs. 3–5. The results showed that the absorbance decreased as indicated by a color change from purple to yellow, as the radical was scavenged by anti radicals.

Table 3 shows that the percentage inhibition of various flower extracts from *A. uliginosa* increase with an increase in concentration of the working solution.

The DPPH is a stable free radical red in color and has an absorbance band at 515 nm. If free radicals have been scavenged by an antiradical compound, DPPH will change color to yellow, which also causes its absorption to disappear. The DPPH has a lone electron which causes a strong absorption maximum at 515 nm, when

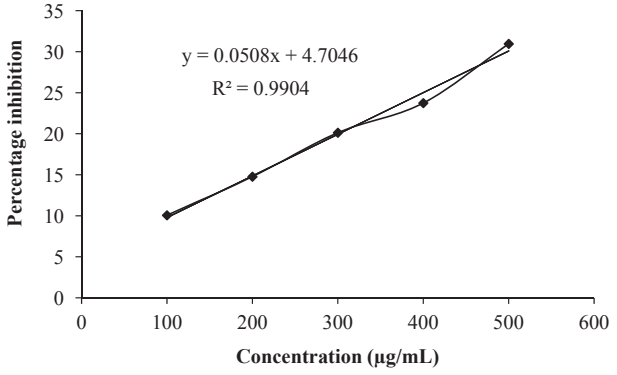

Fig. 5. Comparison of % inhibition of 1,1, diphenyl-2-picryl hydrazine at different concentrations in hexane extract (equation shown by dotted thin line; R^2 = coefficient of determination).

Table 3
Percentage inhibition of 1,1, diphenyl-2-picryl hydrazine of *Acmella uliginosa* (Sw.) Cass in various flower extract concentrations.

No.	Extract concentration (μg/mL)	Inhibition (%)			Hexane concentration (μg/mL)	Inhibition (%)
		Methanol	Ethyl acetate	Hexane		
1	20	14.13	12.20	NA	100	10.07
2	40	21.29	19.40	NA	200	14.77
3	60	32.25	27.96	NA	300	20.13
4	80	40.76	32.51	NA	400	23.74
5	100	50.72	42.17	NA	500	30.96

NA = Not applicable, related to less activity of hexane extract.

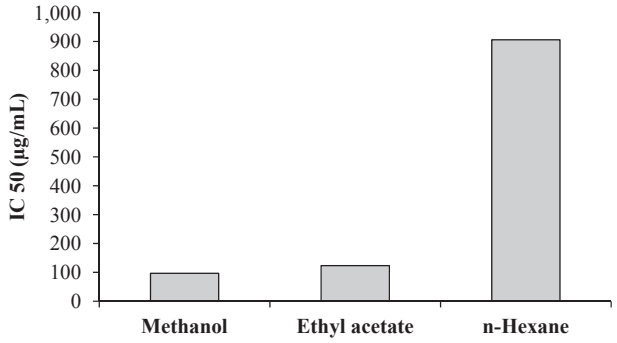

Fig. 6. Percentage inhibiting concentration at 50% response (IC_{50}) from flowers of *Acmella uliginosa* (Sw.) Cass.

this lone electron is paired with another electron from an antioxidant, the absorption strength decreases causing a change in color from red to yellow (Msagati, 2013).

One such method that is currently popular is based upon the use of DPPH. The purpose of this paper was to examine the parameter IC_{50}. whose value was calculated from the plotted graph of scavenging activity against the concentration of the samples.

The DPPH radical scavenging activity in methanol extract and ethyl acetate extract from *A. uliginosa* was recorded in terms of the percentage inhibition. The results showed that the absorbance decreased as a result of a color change from purple to yellow, as the radical was scavenged by anti radicals, through the donation of hydrogen atoms to give a reduction to DPPH-H. The linear regression results are shown in Figs. 3–5. The regression equations were used to determine the IC_{50}.

The ability of *A. uliginosa* in different extract concentrations to donate a proton to a DPPH free radical was assessed in this assay. The concentration of extract scavenging 50% of DPPH radical is shown in Fig. 6. *A. uliginosa* in the methanol extract is a potent antioxidant (IC_{50} = 96.83 μg/mL) compared to the ethyl acetate extract (IC_{50} = 123.46 μg/mL) and the hexane extract (905.92 μg/mL).

Conflict of interest

The authors declare that they have no conflict of interest.

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